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Effects of Acid and Metal Solutions on Seedling Foliage of Two Western Conifers

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Abstract—A greenhouse study tested the effects of three acids and five metals on foliage of lodgepole pine (*Pinus contorta* var. *latifolia*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings. The seedlings were treated with a single immersion of foliage into solutions of three acids (HCL, H_2SO_4, and HNO_3) and five metal chlorides ($ZnCl_2$, $CdCl_2$, $HgCl_2$, $CuCl_2$, and $PbCl_2$), each at five different concentration levels. The treatment levels were pH 6.0, 5.0, 4.0, 3.0, and 2.0 for acids and 0, 0.0001, 0.001, 0.01, and 0.1 moles/liter of each metal chloride. The control treatment for both acids and metals was pH 6.0 distilled water. Injury to the foliage was recorded after 5 weeks by counting needles that were chlorotic (yellow) or dead.

Statistically significant ($p < 0.05$) effects were observed for both acids and metals. The effects of metals were far greater than the effects of acids for both species. Only Douglas-fir seedlings were significantly injured by acids and then only by H_2SO_4 and HNO_3 at their highest concentration (pH 2.0); none of the acid treatments significantly affected lodgepole pine. Zinc, copper, cadmium, and mercury at concentrations of 0.01 molar and stronger significantly affected the foliage of both species. Lead (Pb), however, did not affect lodgepole pine and significantly affected Douglas-fir only at the highest concentration tested, 0.1 molar.

Keywords: lodgepole pine; Douglas-fir; air pollutants; heavy metals; acid rain

Injury to terrestrial ecosystems from airborne pollutants, including acids and metals, has been a concern for many years (Legge and Krupa 1986). Simulated acid rain at pH 1.0 to 3.0 injures the foliage of some conifers (Abouguendia and Baschak 1987; Haines and others 1980; McColl and Johnson 1983). And, in areas where pollutant emissions are high, acidic cloud water is blamed for declines in forest health; the best example is the red spruce (*Picea rubens* Sarg.) decline at high elevations in the northern Appalachians (Hertel and McKinney-McNeal 1991; Siccama and others 1982; Vogleman 1982). If atmospheric acid is responsible for forest decline, it is still not clear whether the primary damage is through foliage (air) or root (soil) systems. In addition, while acid pollution has been relatively well studied in the industrial, populous Northeastern United States, we know little about the effects of acids on Rocky Mountain conifers (Hertel and McKinney-McNeal 1991).

In the Rocky Mountains, metals may be more serious airborne pollutants than acids, because smelters are distributed throughout the region. While metals have negative effects when applied to conifer roots (Patterson and Olson 1983), we found no studies of metals absorbed through conifer needles. Since metals entering the free space of angiosperm leaves damaged leaves (Chino 1981; Hughes and others 1980), we expected conifer needles would be damaged if metals entered them.

We conducted a greenhouse study to learn the sensitivity of Rocky Mountain conifer seedlings to airborne acids and metals. The study also helped in designing a parallel experiment to determine acid-metal effects on trees in the field. We experimentally applied acid and metal analogs of airborne pollutants to determine their effects on seedling foliage. Lodgepole pine (*Pinus contorta* var. *latifolia*) and Douglas-fir (*Pseudotsuga menziesii*), two of the most widespread Rocky Mountain conifers, were studied. This paper reports the extent of the needle injury observed 5 weeks after the acid and metal treatments.

Methods

Trays of 1-year-old lodgepole pine and Douglas-fir seedlings in plastic tubes (2.5 centimeters diameter by 15 centimeters long) were obtained from the U.S.

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Department of Agriculture, Forest Service. The seedlings were from seed sources currently being used by the Forest Service on lands in the area of the aforementioned planned experiment to determine acid-metal effects on trees in the field. They were acclimatized for 90 days in a greenhouse under fluorescent lights with a daily cycle of 15 hours of light and 9 hours of darkness. Greenhouse temperatures were controlled within the range of 10 to 30 °C, averaging 21 °C during the day and 16 °C at night. The seedlings were watered with tap water three times a week.

Acid and metal effects were evaluated with two experiments, one for each species. The species were studied separately because the pine seedlings had a tendency toward chlorosis (yellowing) and a slight degree of needle mottling before the treatments, while the Douglas-fir seedlings appeared uniformly dark green.

In each experiment, five seedlings of a species were randomly assigned to each of 15 acid by concentration treatment combinations, and to each of 25 metal by concentration treatment combinations. Acids tested were HCl (hydrochloric acid), H_2SO_4 (sulfuric acid), and HNO_3 (nitric acid). Metal solutions tested were $ZnCl_2$ (zinc chloride), $CuCl_2$ (copper chloride), $CdCl_2$ (cadmium chloride), $HgCl_2$ (mercuric chloride) and $PbCl_2$ (lead chloride). Concentrations ranged from pH 6.0 (control) to pH 2.0 for acids and from 0 (control) to 0.1 moles for metal chloride solutions. In each case the control was pH 6.0 distilled water. The different acid concentrations were obtained by adding the appropriate amount of pure reagent to distilled water. The metal chloride concentrations were obtained by serially diluting a 1 molar concentration of each metal chloride and distilled water solution to achieve solutions having the desired molarities. A 1 molar solution has the molecular weight of the solute in grams per liter of solution. Each metal chloride concentration was adjusted to pH 6.0, the same as the distilled water control, by adding HCl or NaOH (sodium hydroxide).

We emphasize several details of the procedure used to ensure that only the aboveground portion of the seedlings would be exposed to the treatment and to minimize the possibility of contamination and dilution. Before treatment, the aerial aboveground portion of the seedlings was dipped in distilled water to remove any material adhering to stems and needles, and shaken to remove excess water. The aerial portion of each seedling was swirled in a beaker of its assigned treatment solution for 5 seconds, the solution was shaken off, and then the seedling was swirled in a second beaker of the same solution for another 5 seconds. This process minimized any dilution of the final (second) treatment by water from the cleansing rinse and allowed the foliage to be treated without affecting the roots. After treatment, the seedlings were held upside-down until dry to prevent the solution from running into the rooting medium. When dry, the seedlings were righted and returned to trays in the greenhouse for observation. We avoided contaminating the roots during watering by immersing the tubes in tap water until the soil was saturated; water was delivered to the roots without contacting either stems or leaves.

Needle injury developed gradually after treatments. After 5 weeks differences were visible among the treatments. At that time the total number of needles and the number of injured needles—those exhibiting chlorotic banding, tip chlorosis, or death—were recorded for each seedling. The number of injured needles (sum of banded, tipped, and dead needles) was expressed as a percentage of the total needles for each seedling and used as the dependent variable in statistical analyses.

Data were analyzed by analysis of variance (ANOVA), using the General Linear Models procedures of the SAS statistical software (SAS Institute 1987) to handle imbalance due to several missing values and to provide pairwise comparisons. The data from the two species were analyzed separately due to the aforementioned difference in needle appearance prior to treatments. Because raw data values of percent damage were not normally distributed, they were transformed with the ranking procedure of the SAS statistical software. Ranked values of the dependent variable were used in all analyses of variance and were the basis of all statistical tests reported here. However, means of the raw data were used to indicate actual numerical differences among the various effects. Probabilities of difference of least squares means estimates were obtained from the SAS General Linear Models procedure. These were used for pairwise comparisons to determine which concentrations of acids and metals were significantly different from their distilled water controls. Comparisons of acids across different concentrations and metals across different concentrations were avoided, as were comparisons of acids and metals of different concentrations.

Results

The analyses of variance indicated the following:

- Highly significant differences ($p < 0.01$) between acid treatments and metal treatments for each species
- Highly significant differences ($p < 0.01$) among treatment concentrations for metal treatments on both species
- Significant differences ($p < 0.01$) among concentrations for acid treatments of Douglas-fir, but not of lodgepole pine.

Results of pairwise comparisons to the controls are summarized in the following sections on acid and metal effects.

Table 1—Effect of acids, at different concentrations, compared to the distilled water controls, on lodgepole pine and Douglas-fir seedlings. Values are mean percentages of chlorotic (yellow) and dead needles (standard deviations are in parentheses). Asterisks following data indicate the significance of concentration effects compared to their controls: ** = $p < 0.01$, and *** = $p < 0.001$

	Treatment concentration				
Acid	**Control (pH 6.0)**	**1 (pH 5.0)**	**2 (pH 4.0)**	**3 (pH 3.0)**	**4 (pH 2.0)**
			Lodgepole pine		
HCL	15 (3.8)	18 (6.1)	14 (7.3)	18 (8.6)	17 (10.8)
H_2SO_4	21 (5.2)	15 (11.2)	19 (9.9)	19 (3.2)	29 (17.7)
HNO_3	16 (4.0)	13 (2.8)	21 (15.8)	15 (6.5)	14 (8.0)
			Douglas-fir		
HCL	2 (4.9)	3 (5.6)	4 (5.3)	1 (1.8)	0 (0)
H_2SO_4	1 (0.7)	1 (0.9)	0 (0)	2 (0.8)	16 (6.9)***
HNO_3	0 (0.6)	0 (0)	0 (0)	0 (0.8)	7 (5.1)**

Acid Effects

Neither species was significantly affected by pH levels above pH 2.0 and none of the acid treatments caused chlorosis on lodgepole pine (table 1). Douglas-fir needles were significantly affected at pH 2.0, but only by H_2SO_4 and HNO_3. The percentage of Douglas-fir needles injured by the pH 2.0 concentration was 15 points higher than the distilled water controls for H_2SO_4 and 7 points higher for HNO_3 (table 1).

Metal Effects

The foliage of lodgepole pine and Douglas-fir seedlings was affected by the metal chlorides at 0.01 molar concentration and stronger, with the exception of $PbCl_2$ (table 2). Cadmium chloride also significantly affected Douglas-fir at the 0.001 molar concentration. In general, for both species, foliage's sensitivity was least for lead and most for mercury. Lead chloride had no statistically significant effects on lodgepole pine and affected Douglas-fir only at 0.1 molar concentration. Specific metals appeared to affect Douglas-fir and lodgepole pine differently (table 2). However, we could make no valid statistical test for between-species differences because we did not quantify needle condition before treatment.

Discussion

Acid Effects

Since rainfall's acidity is usually less than pH 3.0 (Knapp and others 1988), we deduce that an acidic rain shower is unlikely to affect conifer seedlings; in

Table 2—Effect of metal chlorides, at different concentrations vs. distilled water controls, on lodgepole pine and Douglas-fir seedlings. Values are mean percentages of chlorotic (yellow) and dead needles (standard deviations in parentheses). Asterisks following data indicate the significance of concentration effects compared to their controls: * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$

	Treatment concentration				
Metal chloride	**Control (0 molar)**	**1 (0.0001 molar)**	**2 (0.001 molar)**	**3 (0.01 molar)**	**4 (0.1 molar)**
			Lodgepole pine		
$HgCl_2$	22 (19.3)	22 (11.8)	17 (6.8)	50 (22.9)**	96 (5.5)***
$CdCl_2$	16 (7.6)	14 (9.1)	21 (8.2)	53 (16.1)***	89 (10.3)***
$CuCl_2$	26 (8.8)	14 (8.4)	29 (15.5)	71 (7.9)***	54 (26.0)**
$ZnCl_2$	16 (5.0)	17 (11.2)	17 (7.3)	35 (9.3)**	74 (16.5)***
$PbCl_2$	11 (8.7)	7 (2.5)	28 (23.8)	14 (9.3)	18 (12.4)
			Douglas-fir		
$HgCl_2$	7 (13.7)	1 (1.2)	1 (1.1)	21 (14.5)**	83 (16.2)***
$CdCl_2$	0 (0)	1 (0.9)	16 (31.6)**	5 (4.2)*	13 (8.5)**
$CuCl_2$	2 (3.2)	0 (0.5)	6 (7.3)	11 (10.7)*	20 (15.6)**
$ZnCl_2$	1 (2.0)	3 (6.9)	1 (1.7)	5 (2.6)**	42 (19.2)***
$PbCl_2$	1 (2.3)	3 (4.8)	1 (0.7)	1 (1.1)	9 (8.2)*

our experiments solutions had to be strongly acidic (pH less than 3.0) to injure Douglas-fir seedlings, and even more acidic (pH less than 2.0) to injure lodgepole pine seedlings (table 1). Our observations are consistent with the finding that needle injury appeared in white pine (*Pinus strobus*) only when acidity increased to pH 1.0 and stronger (Haines and others 1980). Foliage of older trees growing in forests may be even less susceptible to injury by an acid rain event because their needles have thicker cuticles (that may restrict penetration by pollutants) and are less wettable (due to lower contact angles), than those of seedlings—especially those grown in a greenhouse (Shriner 1986).

Because the literature shows that repeated acid applications are injurious to other species, recurring acid rain events might increase damage to lodgepole pine and Douglas-fir. For example, repeated acid applications at pH 2.6 had no effect on jack pine (*Pinus banksiana*), a close relative of lodgepole (Mirov 1956), but caused some bleaching in white spruce (*Picea glauca*). Repeated applications at pH 2.0 seriously injured ponderosa pine (*Pinus ponderosa*), Douglas-fir, and white spruce needles (Abouguendia and Baschak 1987; McColl and Johnson 1983). These differences could be due to differences in exposure time, or differences in elapsed time from treatments to measurement of effects among the various studies. Seedling growth appears more sensitive to acids than needle health and can be affected by acids with a pH of 4.6 (Percy 1986). Occasional reports indicate beneficial effects of low-intensity acid events. In short-term experiments, repeated applications of dilute H_2SO_4 or HNO_3 solutions (pH 2.3 to 5.6) sometimes stimulated the growth of pines, junipers, and Douglas-fir—suggesting sulfur and nitrogen fertilization (Lee and Weber 1979; Wood and Borman 1971). Similarly, increases in photosynthesis have been reported for loblolly pine (*Pinus taeda*) (Lee and others 1990) and red spruce (Kohut and others 1990) needles exposed to pH 3.0 acid rain.

Metal Effects

When metals are absorbed by plant roots (Bazzaz and others 1974; Carlson and others 1975; Chino 1981; Schlegel and others 1987), they can reduce rates of respiration, transpiration, photosynthesis, and growth (Schlegel and others 1987; Van Assche and Clijsters 1983). The injury we observed after foliar application was likely to have reduced at least some of those physiological processes. Electron micrography suggests that chloroplasts, cellular organelles responsible for photosynthesis, are especially sensitive to metals (Guderian 1986).

Since each metal chloride, with the exception of $PbCl_2$, produced significant needle injury in our study (table 2), we expect that repeated foliar exposure to metals can injure conifer seedlings in general.

We expected toxicity at lower concentrations for some metals; for example, mercury, cadmium, and lead are generally more toxic to protoplasm than copper and zinc (Chino 1981; Taylor and others 1990). Instead, metal toxicity generally occurred at a uniform concentration of 0.01 molar in our study (table 2). This suggests that some barrier—likely the cuticle—is finally saturated at a 0.01 molar concentration, allowing the metal to be absorbed and poison the cells within. If so, the surprisingly low needle injury we observed from lead chloride treatments may be due to this relatively insoluble ion's having a superior capability to bind to the cuticles of conifer needles, especially those of lodgepole pine. While copper is generally less toxic to plant tissue than lead, copper did more damage to conifer needles. This may be because copper is especially soluble, enabling it to penetrate conifer cuticles more easily.

Because the metal concentrations that affected foliage in our study are generally stronger than the 0.001 to 0.0001 molar concentrations found in precipitation falling in highly polluted urban-industrial areas (Jeffries and Snyder 1981), single acid rain events should not damage seedlings. However, accumulation of the metal on the needles or in soils could aggravate the problem. At the extreme, if all the metal in acid rain accumulated in or on seedlings, 10 to 100 showers could create deposits sufficiently concentrated to cause foliage injury comparable to that produced by our single exposures. Even if metals do not accumulate on foliage, metals from airborne pollution could accumulate in the soil, where they may injure roots—especially if other pollutants acidified the soil (Smith 1991).

We conclude that seedlings of lodgepole pine and Douglas-fir are not likely to be damaged by single events of acid or metal air pollution. However, repeated exposures may result in metals accumulating in foliage or soils, poisoning these Rocky Mountain conifers. Any acid pollution is likely to increase metal poisoning by facilitating the entry of metals into these conifers.

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